REMARKS

This is meant to be a complete response to the Office Action mailed November 4, 2002. In the Office Action, restriction to one of the following inventions was required under 35 U.S.C. 121:

I. Claims 1-2, drawn to a method for production of multiple Class I HLA antigens comprising applying a PCR reaction to a mammalian DNA specimen and blunt end cloning the PCR product, classified in class 435, subclass 69.3.

II. Claim 3, drawn to a method for production of multiple Class I HLA antigens comprising applying a PCR reaction to a mammalian DNA specimen and cloning the PCR product that has a tail, classified in class 435, subclass 69.3.

III. Claim 4, drawn to a method for production of multiple Class I HLA antigens comprising isolating RNA, synthesizing the first strand of cDNA, and applying a PCR reaction to the cDNA and cloning the PCR product that has a tail, classified in class 435, subclass 69.3.

Applicants respectfully traverse the restriction. However, for the sake of expediting issuance of a patent from the subject application, Applicants elect the invention of Group I, recited in claims 1-2. Claims 3-4 have been canceled herein.

CONCLUSION

This is meant to be a complete response to the Office Action mailed November 4, 2002. Applicants respectfully submit that claims 1-2, as now amended, and newly added claims 5-8 are patentable over the art of record. Favorable action is respectfully solicited.

Should the Examiner have any questions regarding this Amendment, or the remarks contained herein, Applicant's agent would welcome the opportunity to discuss such matters with the Examiner.

Respectfully submitted,

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REDLINE VERSION OF THE CLAIMS

1. (Once Amended) A method for production of [multiple] large quantities of an individual Class I [human leukocyte antigens] MHC molecule, comprising[;] the steps of:

[a)] isolating [a mammalian DNA specimen,] MHC allele mRNA from a source and reverse transcribing the mRNA to form MHC allelic cDNA;

[b) using a PCR reaction with] amplifying the MHC allelic cDNA by PCR using a pair of flanking [oigonucleotide] oligonucleotide primers [and truncating primers] designed to amplify a [first] segment of [said] DNA [specimen] that [codes for] encodes an individual Class I [of a major histocompatability complex (MHC)] MHC gene [,] and truncates said Class I MHC gene by removal of those regions that encode transmembrane and [cytoplastic] cytoplasmic domains of said class I MHC molecules, thereby producing a PCR product that encodes an individual, soluble Class I MHC molecule;

[c) sequencing a DNA product from b) to confirm the identity and fidelity of said truncated molecules,(an automatic sequencer may be used)

d) taking said truncated molecule and] cloning [it] the PCR product into a mammalian expression vector[, and growing by use of normal replication means,] to create a construct;

[e)] electroporating or transfecting [said expression vector with said truncated Class I molecule into a human immortalised cell line,] the construct

into a suitable host cell; and

[f) growing product from e) and screening for most active HLA producing cells by limiting dilution and ELISA assay and starting from one molecule growing cells of said good producers by conventional tissure culture methods, harvesting and pell&mg said cells and resuspending in fresh media;and] inoculating a hollow fiber reactor unit with [said media;

g) feeding said fresh media into said hollow fiber bioreactor unit for continual quantity production of secreted Class I HLA cells into which said Class truncated molecule has been transfected;

i)producing multiple other HLAs by transfecting other Class I alleles into said immortalised cell line and repeating steps b) through g)] the host cell containing the construct such that large quantities of the soluble individual Class I MHC molecule are produced.

2. (Once Amended) [A] The method [for production of multiple Class I human leukocyte antigens as in Claim I)] of claim 1 wherein [said] fresh media, oxygen and glucose are [was] fed into said hollow fiber bioreactor [units] unit at a rate to maintain optimum cell growth [with oygen and glucose levels delivered to said cells in said fresh media with] and to maintain harvest rates [maintained] at a desired level of soluble individual Class I [antigens] MHC molecules.

EXPRESS MAIL NO.: EV 291753153 US **PATENT**
DATE DEPOSITED: MARCH 26, 2003 **ATTY. DKT. NO.: 6680.025**

[TITLE: Equipment and Process for Production of pure major histocompatibility complex (MHC) antigens in human cells for broad Immunology testing and Therapeutic Use]

PRODUCTION OF SOLUBLE HUMAN CLASS I PROTEINS FROM cDNA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] **This application is a continuation of U.S. Serial No. 09/465,321, filed December 17, 1999, now abandoned.**

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

[0002] **At least a portion of the invention was developed under funding from the National Institute of Health ("NIH") under contract Nos. No1-A1-45243 and No1-A1-95360. As such, the Government may own certain rights in and to this application.**

BACKGROUND OF THE INVENTION

[0003] Immunology may be defined as the study of the **body's** reaction to foreign bodies, including microbes[,] as well as macromolecules such as proteins and polysaccarides, without specifying the physiological or pathological consequences of such reactions.

[0004] In the last few years recognition and **[defining]** **definition** of the multitude of genes in the human body **[has]** **have** been proceeding at a rapid rate, and major advances **[as]** **in** the understanding of disease treatment and prevention is occurring.

[0005] The human body has a family of highly polymorphic genes called the major histocompatiblity complex (MHC) which encode **[trausmembrane]** **transmembrane** proteins. Class I and class II MHC molecules play a central role in most if not all adaptive immune responses because class I and class II **MHC** molecules present antigens to T lymphocytes. The human class I and class II **MHC** molecules are known as HLA (Human Leukocyte Antigen) molecules.

[0006] Aside from their central role in directing immune responses against pathogens, the MHC class I and class II molecules are distinguished by their extensive polymorphism. This extensive polymorphism which leads most individuals to be immunologically different, is what causes tissue or transplant rejection between individuals. Differences in the MHC from one individual to another are also why some individuals are susceptible to infections while others are not. Particular MHC types are associated with autoimmune disorders such as diabetes and arthritis.

[0007] Class I and class II HLA molecules contribute significantly to autoimmunity, transplantation, susceptibility and/or resistance to infectious disease, and cancer. For these reasons a detailed understanding of the

biological role of MHC proteins in immune responses is now being sought. To realize the role of HLA class I and class II molecules in human disease requires HLA class I and class II proteins. Clinical therapies to manipulate or alter immune agents which interact with HLA class I and class II molecules will also require MHC proteins.

[0008] An example of the research tests in which MHC class I and class II molecules are used include:

- MHC-peptide multimers used as immunodiagnostic reagents for disease resistance/autoimmunity
- Assessing the binding of potentially therapuetic peptides
- Elution of peptides from MHC molecules to identify vaccine candidates
- Screening transplant patients for preformed MHC specific antibodies
- Removal of antibodies from a patient

[0009] Because these studies are based around human diseases, MHC molecules produced in humans will most readily lead to accurate research results in humans. MHC molecules produced in humans will also lead to immune based intervention and therapy in the clinic.

[0010] There are several research and clinical tests that MHC molecules can be and are used in. These include clinical crossmatch tests for solid organ and bone marrow transplantation (FACS, ELISA, columns), peptide binding tests

which examine the ability of potentially therapuetic peptides to bind to various MHC class I and class II molecules (reference the work of Sette, Buus, Takiguichi, Ramnennsee), and tests for assessing the nature of immune responder cells which provide disease resistance and which drive autoimmune responses (reference Altman, McMichael).

[0011] At this point in time there is no readily available source of HLA class I or class II molecules. For research tests which require relatively pure class I or class **[lithe]** **II MHC** product can be made in bacterial cells. Such is the case for class I molecules used in research experiments. Although the HLA molecules produced in bacteria will not be glycosylated or have human peptides loaded into them, bacterial production is the only means by which enough class I can be produced in a pure form for experiments. Once produced in bacteria the researchers must then load peptide(s) and light chain onto the bacterial class I heavy chain. Only then can experiments be performed.

[0012] When HLA molecules are required from human cells, laboratories typically grow up large volumes (50-100 L) of static cultures (of cells expressing multiple surface-bound HLA class I molecules) in roller bottles, following which they pellet the cells and perform established immunoprecipitation protocols to recover the multiple HLA class I molecules from cell lysates. Using these procedures, the product obtained is typically in the amount of ~300-500 µg. Note that the product obtained in this manner represents a mixture of the six

different HLA class I molecules expressed **[on]** **by** most cell lines. Interpretation of results can therefore **[n t]** **be difficult because ne cannot** be certain that any particular HLA molecule is responsible for a given result. Only indirect conclusions can be reached from these mixtures.

[0013] **[This patent]** **The present invention** comprises equipment and processes for producing relatively large volumes of pure Class 1 molecules at a reasonable cost.

[0014] Class I human leukocyte antigens (ELLA), which are expressed by and present upon virtually every nucleated cell in the body, bind and display cytoplasmically-derived peptide antigens on the cell surface. The peptides they present are derived from either normal endogenous proteins ('self') or foreign proteins **present within the cell** ('nonself'); foreign proteins include products of malignant transformation or intracellular pathogens such as viruses. Class I molecules thus convey information regarding the internal fitness of a cell to CD8+ cytotoxic T-lymphocytes (CTLs), which are activated by interaction with 'nonself' peptides and lyse or kill the cell presenting them. Lymphocytes are a type of white blood cell or leukocyte that circulate in the lymph.

[0015] HLA class I molecules exhibit extensive polymorphism, which is generated by systematic recombinatorial and gene conversion events; it is for this reason that hundreds of different HLA types exist throughout the world's populations. Most people therefore differ in their MHC class I molecules.

[0016] Transplantation between individuals with different class I and class II MHC molecules leads to the production of strong immune responses. Some of these immune responses can be controlled with drugs. The immune responses that can be controlled with drugs are new immune responses, or immune responses that form after the transplant. In fact, immunosuppresive drugs are now so effective that it is becoming much more common to transplant organs that are not well matched for their MHC class I and class II molecules. However, immunosuppresive drugs cannot stop the rejection of an organ transplant when the organ recipient has circulating antibodies which recognize the organ being transplanted. A number of events can trigger the production of antibodies against MHC class I molecules in other individuals. These events include blood transfusion, pregnancy, bacterial infections, and other less understood events. So, it is not uncommon for an individual who needs a heart, kidney, lung, or liver transplant to have circulating antibodies which would immediately attack some transplanted organs. These circulating antibodies cannot be inhibited with drugs, and transplanting an organ that is recognized by such antibodies will lead to organ failure before the transplant operation is finished.

[0017] **[Let's use Man X as an example. Man X needs a liver. With the powerful immunosuppresive drugs now available the transplant physician can give Man X a liver from almost any donor. The**

immunosuppresive drugs will mean that the liver d n r d es n t have to be well matched because the drugs can keep the immune response from forming an attack against the nonmatched liver. However, if Man X has antibodies that recognize the liver being transplanted then he will reject the liver before it is completely sewn in.]

[0018] [So, in order to insure that Man X does not have antibodies against MHC class I, the hospital will take blood from Man X once a month and test it to see what MHC class I Man X does have antibodies to. If Man X has antibodies to particular MHC molecules in the organ to be transplanted then the physician will be sure not to transplant an organ with those class I molecules into Man X.]

[0019] [How do they do this test? They get Man X's antibodies (easy) and they see what class I molecules his antibodies recognize.] The limiting reagents **[in all of this]** **in determining if a patient is producing circulating antibodies that would attack a transplanted organ** are Class I and Class II pure protein**s** **[antigens]**. There is no good source of Class I and Class II to screen **[Man X's]** **a patient's** antibodies against at present. **[This patent]** **The present invention** is aimed at filling this need for Class **[1]** **I MHC** molecules. **[With advances in immunosuppressor drugs transplant physicians are now poised to do transplants between the organ to be transplanted and unmatched individuals. Less matching in**

now being done between organ donors and recipients than ever bef re. However, with less matching the odds go up that Man X will have antibodies against class I molecules; you only make antibodies against 'foreign' objects, or those that are not matched and therefore different than self. Thus, because] **Because** complete organ matching is becoming less common, **[and our test to look]** **the method of the present invention, including the ability to screen** for preexisting antibodies against the Class I HLAs on organs to be transplanted, is very important.

SUMMARY OF INVENTION

[0020] The **present** invention encompasses **a method for** production of **individual** Class I **[single human leukocyte antigens (HIA) of the polymorphic]** major histocomptability **complex** (MHC) **[of the immune system]** **molecules which are secreted from mammalian cells in a bioreactor unit.**

[0021] Class I **MHC** molecules are ordinarily expressed on the cell in a membrane-bound form; they consist of an extracellular domain, a transmembrane domain, and a short cytoplasmic domain. **[We modify the]** **In one embodiment of the method of the present invention, the DNA encoding the Class I MHC** molecules **is modified** using PCR so that **[they no longer]** **the Class I MHC molecules expressed from the DNA do not**

have transmembrane or cytoplasmic domains; since **[they]** **the Class I MHC pr teins are** no longer **[are]** anchored in **[to]** the membranes of the cells expressing them, they are **soluble and** therefore secreted into the supernatant media surrounding the cells**. [as they grow. We collect the]** **The Class I MHC** molecules produced in this manner **are collected** as 'harvests' from a hollow fiber bioreactor sysem.

[0022] **[Once we have made a]** **A** 'construct' encoding a soluble **Class I MHC** molecule **[(this consists of taking]** **is produced by creating** the truncated PCR product **described above** and placing it in a DNA vector that contains a promoter which is required for expression of the **[molecules), the]** **DNA. The** construct is introduced into a mammalian cell line so that it can be expressed; **[we use]** **for example,** a human B-lymphocyte line which is mutated so that it does not express any class I molecules other than the one coded for in the construct **[that we incorporate into the cell]** **may be utilized, thereby easing purification of the desired Class I MHC molecules since no other Class I molecules are present.** The **[obvious pluses]** **advantages** of this system are that (i) the resultant **soluble Class I MHC** molecules are produced and folded 'naturally' (since they are generated within mammalian cells, rather than made using either bacteria or insect cell lines; we furthermore have published data confirming that the soluble **Class I MHC** molecules appear to bear functional properties identical with those of full-

length, cell surface-expressed HLA molecules), (ii) due to continuous secretion, large quantities of soluble **Class I MHC** molecules can be obtained with relative ease using hollow-fiber bioreactor systems, and (iii) the product yielded is significantly "cleaner" to begin with than the small quantities obtained by traditional cell lysate/immunoprecipitation protocols previously **[touched-upon] described**. Edman sequencing**[,]** and mass spectrometry show that the protein content of the material **[we]** obtain**ed** from **such** harvests consists of soluble HLA molecules of a single**, individual** class I **MHC** molecule.

[0023] The total steps necessary to produce cell lines prior to growth in the hollow fiber bioreactor systems is considered a 'limiting factor' in that it requires multiple factors including (i) subcloning/sequencing to obtain specific constructs, (ii) transfection/screening by ELISA of mammalian cells to obtain cell lines which have taken up the completed DNA vector for expressing the molecule, (iii) limiting dilution subcloning/screening by ELISA to obtain cell lines of maximum soluble HLA production levels, and (iv) RT-PCR/sequencing to validate cell lines before bioreactor culture.

[0024] [In use there is a major advantage using a single MHC molecule in a test or experiment. Experimental results are not clouded by the confounding fact that multiple MHC molecules are present. This is a major advantage over existing tests which rely on mixtures of MHC molecules. Furthermore, the MHC molecules provided here are

produced in human cells. The only existing means f r pr ducing individual MHC molecules is in n n-mammalian cells. Production in human cells means that the MHC molecules produced most resemble human proteins, noting that these proteins will be used in applications for humans. For this reason MHC molecules produced in bacterial or yeast cells are not used for diagnostic or therapuetic purposes. Mixtures of MHC molecules from human cells are now the norm. We will provide] **The Class I MHC molecules produced by the method of the present invention overcome the disadvantages and defects of the prior art in that individual rather than multiple MHC molecules are present, and the individual MHC molecules produced by the method of the present invention more closely resemble human proteins than the individual MHC molecules currently produced in bacterial or insect cells.** **The** individual MHC molecules produced in human cells **[for the following:]** **by the method of the present invention may be utilized for** HLA crossmatching, **[-Absorbtion]** **absorption**/removal of anti-HLA antibodies from patients, **[-Development]** **development** of HLA presented peptide based vaccines, **[-Discovery]** **discovery** of pathogen based peptide epitopes for vaccine use, **[-Characterization]** **characterization** of immune effector cells, **and the like.**

[0025] **[We have documented that our unique system]** **The method f the present invention** produces individual MHC molecules in sufficient quantity for numerous experimental and clinical applications. The MHC molecules are produced from human cells and in all measurable ways [they] resemble surface bound MHC molecules found on healthy human cells. Chaperone interaction, peptide loading, and antibody reactivity are all normal.

DETAILED DESCRIPTION OF THE INVENTION

[0026] **[We will describe the invention by outing steps that we currently use to produce the pure protein antigens from single Class I HLAs.]**

[0027] **[In Step 1 we may start with an EBV (Epstein-Barr virus) transformed cell line]** **The method of the present invention begins with the isolation of a mammalian DNA specimen or source** containing **[the]** multiple alleles or forms of the MHC. **[of a human DNA strand. We start at this point because this]** **For example, an EBV (Epstein-Barr virus) transformed cell line may be utilized as it** is readily commercially available. **[We could start from]** **Alternatively,** normal mammalian DNA **[and produce the]** **may be utilized, or a** virus transfomed cell line or an immortalised cell line **may be constructed** by known methods.

[0028] **[Using PCR we grow the cell line and in Step 2 we spin down**

cells and extract the t tal] **Total** RNA **is isolated fr m the source by kn wn methods, such as by** using Qiagen RNA extraction kit or similar separation means. **The total RNA contains mRNA for the one or more MHC alleles. The mRNA is then reverse transcribed to form cDNA. [In Step 3 cDNA is formed from Step 2 product using an Amersham-Pharmacic Biotech Kit or similar equipment and reverse transcriptase to form complementary DNA or cDNA.]**

[0029] [In step 4 product from Step 3 is treated with a pfu] **The allelic cDNA is then amplified by PCR using *Pfu*** polymerase **[enzyme]** or **other** similar enzyme and **flanking oligonucleotide primers. The** primers **[to cut off a sequence which encodes cytoplastic] are designed to amplify a segment of DNA that encodes a Class I MHC gene and truncates the Class I MHC gene by removal of the regions that encode the cytoplasmic** and transmembrane domains **of the Class I MHC molecule,** thereby truncating **[said molecules] the Class I MHC molecule encoded by the PCR product** so that the normally **[surface expressed] membrane bound** peptides will be secreted in the surrounding solution. **[If we wish] The primers may also be designed to add a tail to the expressed protein to aid in purification thereof. For example, if it is desired for** the secreted peptides to have histidine tails, **[another] a** primer such as 3PEI-His is used. Tails other than **[the histadine tails shoud] histidine tails should**

be equally useful. The truncated **PCR** product may **then** be purified using a Qiagen **[PCRPurification]** **PCR purificati n** kit or similar apparatus**, and the PCR product sequenced to confirm the identity and fidelity of the PCR product.**

[0030] **[In Step 5 restriction enzymes such as EcoRI and XbaI cut the product to form single strands and form]** **The PCR product is then introduced into a mammalian expression plasmid or vector, such as, but not limited to, the pcDNA3.1 vector, by methods well known to a person having ordinary skill in the art, thereby creating the desired construct.**

[0031] **[In step 6 using a T4 enzyme ligase insert the PCR product into the vector.]**

[0032] **[In step 7 transform JM 109, a strain of E-coli, using the ligated product and vector from step 6; thus causing the ligated vector to enter the JM109 bacteria. Other bacteria should work essentially as well.]**

[0033] **[In step 8 plate out the transformed JM109 on 96 cell LB/ampicillin plate or similar equipment and grow for about 12 hours. Only antibiotic resistant colonies should grow.]**

[0034] **[In step 9 pick out antibiotic resistant colonies and grow in LB/ampicillin media while shaking at 300 rpm for approximately 18**

h urs and make a small v lume of glycerol st cks f each and all w t stand vernight.]

[0035] [In step 10 using a portion of the glycerol stock, extract the vector or plasmid containing the PCR product insert using a Promega Wizard mini prep kit. This extraction occurs by the vector sticking to the kit membrane where it is then water washed.]

[0036] [In step 11 using the extracted vector and using restriction enzymes such as CcoR and Xbal carry out said restriction digest on an ethidium bromide agarose electrophororesis gel. The ethdium bromide is an intercalating agent and allows us to visualize the DNA in the gel under ultraviolet light. Grow to bring up the concentration of the vector to 9.5µg/10µl.]

[0037] [In step 12 using step II product and primers such as BGH, 3S, and T7 to cut double DNA strands and using an Amersham Pharinacia Sequencing kit and sequencing gel analyze the data to make certain the insert has no errors. If errors are found go back to the glycerol solutions and repeat the steps.]

[0038] [In step 13 pick a good clone from step 12 and streak an LB/ampicillin plate using the using the stored glycerol stock of bacteria and grow overnight; pick a colony from the plate, place in 45 mls of LB/ampicillin media and grow for 16 hours while shaking at 300 rpm.

Actual timeand shake rate may be varied.]

[0039] [In step 14 using a Qiagen Midi kit r similar kit extract the plasmid containing the PCR product insert from the 45 mls of solution.]

[0040] In step 15 using] **A suitable host cell, such as** cell line 721.221 or an immortalised cell line that lacks expression of Class **[15 MHCs grow using PCR until it is in log phase]** **I MHC molecules is chosen and electroporated or transfected with the construct. The host cells containing the construct are isolated and screened to identify the most active HLA producing cells, and these cells are then used to inoculate media in roller bottles or similar containers where small amounts of individual MHC molecules are desired, or media in a hollow fiber bioreactor unit where large scale continuous production of individual MHC molecules are desired.**

[0041] Described herein below is one Example of the method of the present invention. However, the steps outlined herein below are only provided for the purposes of example only and are not to be construed as limiting.

[0042] In step 1, a cell line containing multiple human MHC alleles was isolated. Total RNA was then extracted from the cell line using Qiagen RNA extraction kit or similar separation means, and the mRNA present in the total RNA was reverse transcribed to form cDNA. This

step is typically perf rmed using a kit, such as a kit produced by Amersham-Pharmacia Biotech, and reverse transcriptase.

[0043] The cDNA encoding the desired MHC Class I allele was then amplified by PCR using *Pfu* polymerase or other similar enzyme and flanking oligonucleotide primers. The primers utilized in the PCR reaction were designed to remove the sequences encoding the cytoplasmic and transmembrane domains of the Class I MHC moleule, so that the resulting PCR product only encodes a secreted form of the Class I MHC molecule. In addition, the primer 3PEI-His was utilized so that the secreted form of the Class I MHC molecule encoded by the PCR product has a histidine tail attached thereto for aiding in purification of the secreted Class I MHC molecules. The truncated PCR product was then purified using a Qiagen PCR Purification kit or similar apparatus.

[0044] To clone the PCR product into the mammalian expression vector pcDNA3.1, the PCR product was cut with the restriction enzymes *EcoRI* and *XbaI* and ligated with the vector using T4 ligase. To ease in sequencing of the construct, the ligated PCR product and vector were transformed into *E. coli* JM-109 and plated on LB-ampicillin plates. The construct was extracted from antibiotic resistant colonies using a Promega Wizard mini prep kit, and the presence of the PCR product was confirmed by restriction enzyme digestion using *CcoR* and *XbaI*.

The PCR pr duct was then sequenced using primers such as BGH, 3S and T7 and an Amersham Pharmacia Sequencing kit to c nfirm the identity and fidelity of the DNA sequence of the PCR product and to make certain the insert had no errors.

[0045] The plasmid containing the PCR product insert was then extracted from 45 mls of solution using a Qiagen Midi kit or other similar kit, and the plasmid or vector containing the PCR product was electroporated or transfected into 721.221 cells. G418 resistant cells were selected and screened for most active HLA producing cells by limiting dilution and ELISA assay. For example, the G418 resistant cells were serially diluted to a point where there was one cell per well, and an ELISA assay was used to determine which cells were producing the most soluble HLA.

[0046] [In step 16 electroporate the plasmid or vector containing the PCR product insert into the 721.221 cells and using G418 or similar antibiotic choose electroporated cells that are resistant to the antibiotic proving the cells contain the vector with the insert therein.]

[0047] [In step 17 using electroporated cells serially dilute in an agarose plate to a point where there is one cell per cup, grow and using ELISA Assay determine which cells are producing the most soluble HLA.

[0048] **[In step 18 gr w]** **The** most prolific cells **were grown** in complete media in roller bottles or similar containers. **[Where nly small amounts are needed the small amounts may be harvested from the roller bottles or similar equipment. Inoculate a hollow fiber reactor unit with the product.]** **A portion of the cells growing in the roller bottles were used to inoculate a hollow fiber bioreactor unit.**

[0049] **[In step 19, product]** **The inoculate** from the roller bottles **[is]** **was** fed counter current to harvest media in the hollow fiber bioreactor unit for continous large scale production of desired secreted **[antigen]** **Class I molecules. The inoculate was fed into the hollow fiber bioreactor unit at a rate to maintain optimum cell growth.** Oxygen, glucose and carbon dioxide **[are]** **were** fed into the temperature controlled circulating stream with feed rate, circulating rate, oxygen, glucose, **[a nd]** carbon dioxide and pH all controlled to **[to]** **provide a** harvest **rate that is maintained at a desired level of soluble Class I MHC, for example,** a concentration of about 3µg/ml of **secreted** class I pure protein **having** antigen **bound thereto may be obtained. The hollow fiber bioreactor units can be used for continuous, large scale production of large quantities of secreted individual Class I HLA molecules.**

ABSTRACT OF THE DISCLOSURE

Starting with DNA, a method for producing large volumes at a reasonable cost of secreted HLA's from a single allelle of Class [1] I MHC's in a human cell line using a hollow fiber bioreactor is disclosed.